Troubleshooting the immunoprecipitation procedure

Problem: Tagged proteins not visible or only weakly visible on Western blot

Possible cause	Remedy
Sample degraded by proteases	Include additional protease inhibitors in lysis and wash buffers.
	Keep sample cold at all times.
Antibody concentration too low	Increase concentration of precipitating antibody.
Antibody has low affinity for tagged protein	Use lower stringency wash buffers (for instance 150 mM NaCl, no detergent).
Precipitating antibody did not bind to protein A-agarose	Substitute protein G-agarose.
Precipitating antibody did not bind to protein G-agarose	Substitute protein A-agarose.
Tag sequence not accessible to precipitating antibody, due to conformation of tagged protein	Use alternative insertion sites within the target gene for the tag sequence.
	Insert multiple tag sequences into the target protein to increase avidity of antibody reaction.
Problems during Western transfer	See "Troubleshooting the Western blot procedure" in Section 3A of this manual.
Antibody or protein (A/G) incubation too short	Incubate with precipitating antibody for several h at 4°C.
	Incubate with protein (G/A)-agarose overnight.

Problem: High background or many nonspecific bands on gel or blot membrane

Possible cause	Remedy
Nonspecific proteins bind to protein (G/A)-agarose or are entrapped in the protein (G/A)-antibody-antigen immunocomplex.	Prepare cell samples in serum-free media. Repeat the preclearing procedure (Procedure IV) several times before doing immunoprecipitation.
	Increase the washing time after immuno-precipitation.
	Increase the stringency of the washes. For example, wash with a buffer containing a higher concentration of detergent or salt.
	During binding and wash steps, let protein (G/A)-antibody-antigen immunocomplex settle by gravity rather than by centrifugation.
	Preload protein (G/A)-agarose with specific antibody, then block remaining sites with nonspecific antibodies; use this protein (G/A)-antibody complex for immunoprecipitation.
Contaminated equipment or buffers	Use clean equipment and freshly prepared buffers.
Foreign material on blot membrane	Do not touch the membrane with bare hands; use powder-free gloves and blunt-ended, non-serrated forceps.

Suggested reading to learn more about procedure

There are numerous published procedures for performing immunoprecipitations. For a detailed description of these techniques and discussion of all important factors affecting the results, see Harlow and Lane (1988).